# Protocol for Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells
**(December 2014)**

## I. Introduction

The preparation and maintenance of key reagents used for the Neutralizing Antibody Assay for HIV-1 in TZM-bl cells is crucial for obtaining accurate and reproducible results. Reagents must be created and stored as per manufacturer's guidelines and must be used within pre-established expiration dates.

## II. Definitions

GM: Growth Medium

DMEM: Dulbecco's Modified Eagle Medium

FBS: Fetal Bovine Serum

HEPES: N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid

DEAE-Dextran: Diethylaminoethyl-Dextran

MSDS: Material Safety Data Sheet

COA: Certificate of Analysis

## III. Reagents and Materials

Recommended vendors are listed. Unless otherwise specified, products of equal or better quality than the recommended ones can be used whenever necessary.

**Growth Medium**

DMEM, with L-glutamine, sodium pyruvate, glucose and pyridoxine,
*Vendor:* Gibco BRL Life Technologies
Sterile, store refrigerated at 4ºC

Fetal bovine serum
*Vendor:* Hyclone
Heat-inactivated 56$^{o}$C for 30 minutes, 500 ml bottle, sterile. Store at −20$^{o}$C. Once thawed, store at 4$^{o}$C.

Gentamicin solution, 10 mg/ml
*Vendor:* Sigma
Sterile, store at 4ºC

HEPES
*Vendor:* Gibco BRL Life Technologies
Sterile, store at 4°C

**DEAE-Dextran, hydrochloride, average Mol. Wt. 500,000**
*Vendor:* Sigma

**Britelite Plus Reporter Gene Assay System**
*Vendor:* Perkin Elmer Life and Analytical Sciences

**15 ml conical polypropylene tubes**
*Vendor:* Generic

## IV. Instrumentation

Recommended manufacturers are listed. Unless otherwise specified, equipment of equal or better quality than the recommended ones can be used whenever necessary.

**Pipettor**
*Manufacturer:* Drummond
*Manufacturer:* Rainin

**Scale**
*Manufacturer:* Mettler

**4°C Refrigerator**
*Manufacturer:* Sci-Cool

**−20°C Freezer**
*Manufacturer:* Sci-Cool

**Low Temperature Freezer (at least −70°C)**
*Manufacturer:* Thermo Labsystems

## V. Protocol

### 1. Growth Medium

**1.1** Complete GM consists of DMEM containing 10% heat-inactivated FBS, 50 μg gentamicin/ml, and 25 mM HEPES.

**1.2** To make 500 ml of Complete GM, combine and mix in a sterile bottle:

435 ml DMEM

50 ml FBS

2.5 ml of gentamicin

12.5 ml of HEPES

**1.3** Store the Complete GM at 4°C for up to 2 months (or to the earliest expiration date of any one of the constituent reagents, whichever comes first).

**1.4** Before use in the assay, warm medium to 20°-37°C.

## 2. Antibiotic-free Growth Medium

**2.1** Antibiotic-free GM consists of DMEM containing 10% heat-inactivated FBS.

**2.2** To make 500ml of antibiotic-free GM, combine and mix in a sterile bottle:

437.5 ml of DMEM

50 ml of FBS

12.5 ml of HEPES

**2.3** Store the antibiotic-free GM at 4°C for up to two months (or to the earliest expiration date of any one of the constituent reagents, whichever comes first).

**2.4** Before use in the assay, warm medium to 20°-37°C.

## 3. DEAE-Dextran

**3.1** To prepare a 7.5 mg/ml solution, dissolve 3.75 gm of DEAE-Dextran in 500 ml of sterile water.

**3.2** Create 10 ml aliquots in 15 ml sterile conical polypropylene tubes.

**3.3** Store aliquots at −80°C.

**3.4** DEAE–Dextran from some manufacturers does not have a listed expiration date. Contact the manufacturer for the stability of each DEAE–Dextran lot.

***NOTE 1:*** It is important to note that conical tubes with DEAE-Dextran solution should not be placed into styrofoam racks for storage until after contents are completely frozen.

The freezing process begins at the exposed part of the tube. Consequently the shielded bottom of the tube will crack rendering the contents of the vial unusable.

## 4. Britelite Plus Reporter Gene Assay System

**4.1** Reconstitute one bottle of lyophilized Britelite Plus Substrate Solution with the Britelite Plus Substrate Buffer Solution (as per manufacturer's instruction).

**4.2** Mix the contents of the vial by inversion until the substrate is completely dissolved (about 1 minute).

**4.3** Distribute 10.5 ml to 15 ml conical polypropylene tubes and store at −20°C for 1 month or −80°C for 3 months (or until the expiration date of the reagent, whichever comes first).

**4.4** Thaw in a room temperature water bath in the dark immediately before each use.

**4.5** Mix gently prior to use. Use within 60 minutes of thawing. Excess reagent may be stored at −20°C or −80°C and used a maximum of 10 freeze/thaw cycles.

***NOTE 2:*** The lyophylized Britelite Plus substrate is not classified as hazardous.

*Bright Glo substrate solution from Promega and Britelite substrate solution from Perkin Elmer Life and Analytical Sciences are acceptable substitutes for Britelite Plus. Please follow manufacturer's guidelines for preparation and use. Britelite and Bright Glo are classified as hazardous. Personal Protective Equipment (PPE) is required when working with these reagents.

***NOTE 3***: All reagents must be stored according to manufacturer's specifications and within manufacturer's specified expiration dates. An up to date MSDS and COA must be retained in the laboratory.